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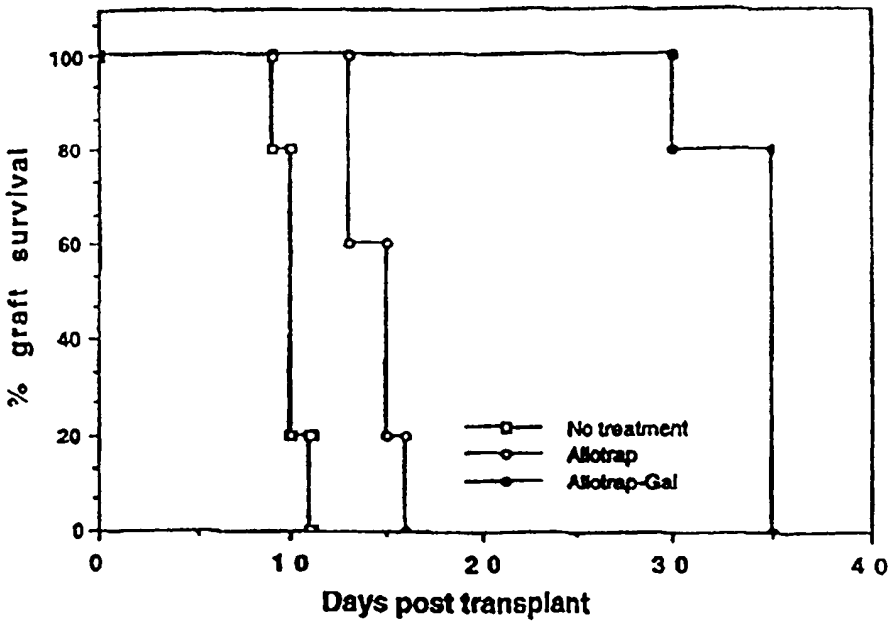

(57) Abstract

Novel methods for enhancing the effective in vivo half life of therapeutic compounds in mammals are provided. Specifically, therapeutic compounds are conjugated to a haptenic moiety. Upon administration to the mammalian subject, the therapeutic compound/hapten conjugates are bound by circulating antibodies directed specifically against the haptenic moiety of the conjugate, thereby resulting in a stabilization of the conjugate in the vascular system and an effective increase in the in vivo half life of the therapeutic compound.

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ENHANCED EFFECTS FOR HAPTEN CONJUGATED THERAPEUTICS

Cross-Reference to Related Applications

This application is a continuation-in-part of U.S. Patent application Serial No. 08/630,383, filed April 10, 1996, which is a continuation-in-part of U.S. Patent application Serial No. 08/254,299, filed June 6, 1994, which is a continuation-in-part of U.S. Patent application Serial No. 07/690,530, filed April 23, 1991.

Technical Field

The field of this invention is modification of the pharmacokinetics and pharmacodynamics of drugs or biologicals in vivo, specifically, for enhancing the effective in vitro half-life of therapeutic compounds in mammals.

Background

Many therapeutic compounds which are used for the treatment of human disease show marked efficacy in controlled in vivo systems. However, in a large number of cases, the ability of these compounds to produce long-term therapeutic effects in vivo is greatly reduced because their survival in the circulation may be limited to a very short period of time. As a result, the attending physician often has no recourse but to increase the dosage and/or frequency of drug administration to achieve the desired therapeutic effect. Clearly, however, increasing the dosage and/or frequency of administration is a detriment to the patient

because it increases the complexity and the cost of treatment and may result in toxic side effects associated with increased doses of the therapeutic compound.

There are a number of diverse methods that have been suggested and implemented for effectively increasing the in vivo half-life of biologically active therapeutic compounds. Many of these involve the physical entrapment of drugs by the fashioning of unique dosage forms that include enteric coatings, microencapsulation, and the like, thereby resulting in the sustained release of active therapeutic compound over time. Another example involves the covalent linkage of various components to the drug of interest prior to administration. For example, the use of such components as polyethylene glycol as a drug carrier is well known, however, such modification often results in a drug/carrier complex that is immunogenic in mammals, thereby resulting in the induction of an immune response and clearance of the complex from the system.

Therefore, there exists a need for methods useful for enhancing the effective half-life of biologically active therapeutic compounds in vivo. An increase in the effective in vivo half-life of such compounds would result in enhanced efficacy of therapeutic treatment as well as a decreased effective dose for products which exhibit toxic side effects. Furthermore, this is accomplished within a strategy that is not limited by the induction of a patient's immune response against the enhanced therapeutic agent.

The use of non-immunogenic "Xenoject" compounds fulfills these criteria resulting in a novel strategy for improving patient care. A "Xenoject" compound is a conjugate of a therapeutic compound and a haptenic moiety which, when administered to a mammalian host, is recognized and bound by circulating antibodies directed against the haptenic moiety, thereby effectively increasing the in vivo

half-life of the therapeutic compound in the vascular system.

Relevant Literature

Sato et al., *Biotherapy* 6:225 (1993), Martens et al., *Eur. J. Immunol.* 23:2026 (1993), and Suzuki et al., *J. Immunol.* 152:935 (1994) describe the prolongation of the serum half-lives of interleukin-2 or -6 when bound to anti-interleukin monoclonal antibodies in the serum. Galili, U., *Immunol. Today* 14:480 (1993) reviews the discovery of the anti-αGal natural human antibody response. Lussow et al., *J. Immunother.* 19:257 (1996) and Lussow et al., Transplantation 62:in press (1996) describes the redirection of circulating antibodies for cell killing by complement activation. Thall et al., *J. Biol. Chem.* 270:21437 (1996) presents the development of α-galactosyltransferase knock-out mice. Ono and Lindsey, *J. Thorac. Cardiovasc. Surg.* 7:225 (1969) teach a surgical procedure for the heterotopic transplant of hearts between rodents.

Clayberger and Krensky, *Curr. Opinion Immunol.* 5:644 (1995) review the genesis of the HLA derived immunosuppressive Allotrap peptides. Nossner et al., *J. Exp. Med.* 183:339 (1996) suggests that Allotrap peptides bind to the hsp 70 protein. Gao et al., *Immunosuppression* 15:78 (1996) evaluates the serum protease resistance of D-Allotrap peptides with respect to L-Allotrap peptides.

Summary of the Invention

Therapeutic compounds are conjugated to haptenic moieties that are recognized and bound by circulating antibodies in vivo. The therapeutic compound/hapten conjugates, referred to herein as "Xenoject compounds", have an increased effective in vivo half-life as compared to that of the unconjugated therapeutic compound. The increase in in vivo duration is due, at least in part, to binding of the haptenic moiety of the Xenoject compound to the antigen

binding site of a circulating antibody, thereby resulting in a stabilization of the drug in the serum. The drug then remains in the circulation with a half-life more closely resembling that of an immunoglobulin molecule rather than of a free small molecule. This allows lower doses of the therapeutic to be used which is of particular benefit when the compound is inherently toxic.

Brief Description of the Drawings

Figure 1 illustrates the enhanced effects of binding a therapeutic compound to circulating antibodies via a haptenic moiety. Transplanted α-galactosyltransferase knock-out mice, each of which had high-titers of circulating anti-α-Gal antibodies, were treated for 10 days with 1 mg/Kg of D-Allotrap/α-Gal conjugate (●), unconjugated D-Allotrap (O), or no peptide (□). Cardiac graft survival was measured by direct palpation of the hearts through the peritoneum. Results were plotted as the percent graft survival over time after transplantation.

Description of the Specific Embodiments

In accordance with the subject invention, methods are provided for prolonging the effective in vivo half-life of a therapeutic compound in a mammal. The herein described methods for prolonging the effective in vivo half-life of a therapeutic compound in a mammal comprise conjugating the therapeutic compound to a haptenic moiety to provide a therapeutic compound-hapten conjugate (herein also referred to as a "Xenoject compound"). Upon administration of the therapeutic compound-hapten conjugate to a mammal, the haptenic moiety of the conjugate is recognized and bound by a circulating antibody present in the mammal which is directed against that hapten, thereby serving to stabilize the conjugate in the mammalian vascular system. The therapeutic compound thereby exhibits an effective half-life that more closely resembles that of an immunoglobulin

molecule rather than that of a free small molecule in the vascular system.

The effective in vivo half-life of numerous different therapeutic compounds can be prolonged by employing the novel methods described herein. In this regard, the phrases "therapeutic compound", "drug" or grammatical equivalents thereof refer to any compound that, when administered to a mammalian subject, gives rise to a desired therapeutic effect. Therapeutic compounds that find use in the presently described methods include, for example, peptides, including for example, Allotrap peptides and cyclosporines, peptidomimetics, proteins, including for example, immunoglobulins or therapeutically effective fragments thereof, hormones, including for example estrogens, progesterones, growth hormone, and the like, enzymes, enzyme inhibitors, interleukins, including interleukin-2, cytokines, growth factors, nucleic acids, chemical compounds, including organic compounds, metallic compounds, chemotherapeutic compounds, and the like, and radioactively labeled compounds. For the most part, the therapeutic compound is not critical to the invention in that virtually any therapeutic compound can be successfully conjugated to a hapten provided that the compound possesses a site at which the hapten can be conjugated without substantially affecting the therapeutic activity of the compound.

Therapeutic compounds which find use in the methods of the present invention may be naturally occurring or synthetic. Such compounds may also be "biologically active" in their native state, meaning that the compound itself possesses the ability to provide a desired therapeutic effect without any modification of that compound. On the other hand, therapeutic compounds that find use may also be biologically inactive or in a latent precursor state when administered as part of a therapeutic compound/hapten conjugate, but may acquire biological or therapeutic activity when a portion of the therapeutic compound is

hydrolyzed, enzymatically cleaved or is otherwise modified in the mammalian vascular system or at the specific target site. In this regard, the therapeutic compound may be a "pro-drug", meaning that the compound is essentially therapeutically inactive when administered but becomes active upon modification in the vascular system. For example, specific hydrolyzable groups may be attached to therapeutic compounds by methods known in the art, said hydrolyzable groups being hydrolyzed after administration of the conjugate to the mammal, thereby resulting in activation of the therapeutic compound. Moreover, the essentially inactive therapeutic compound may be enzymatically cleaved or otherwise modified in the vascular system to provide for the biologically active compound. An example of such a precursor therapeutic compound is, for example, pro-insulin. The pro-drug may be modified in the vascular system over time to provide a large depot of active drug or may remain inactive until it reaches a specific target site. Once activated by hydrolysis, enzymatic cleavage or by other modification, the therapeutic compound may act at the surface of a target cell or may be transported into the target cell to act intracellularly.

By "prolonging the in vivo half life of a therapeutic compound", "stabilizing" a therapeutic compound or grammatical equivalents thereof is meant that the in vivo half-life of a therapeutic compound when conjugated to a haptenic moiety is increased relative to the in vivo half-life of the same therapeutic compound that is not conjugated to a haptenic moiety. Techniques for determining the in vivo half life of therapeutic compounds are well known and conventionally used in the art and include, for example, determining the presence and/or activity of the therapeutic compound in the vascular system over time.

Haptenic moieties that find use for conjugation to a therapeutic compound of interest are specifically recognized and bound by circulating antibodies present in the mammalian

vascular system and include, for example, xenoantigens, including, for example, sugar moieties typically found on glycoproteins such as Gal α1-3 Gal and mimetics of those sugar moieties (e.g., see Vauehan et al., *Xenotransplantation* 3:18-23 (1996)), blood group antigens, and the like. Haptenic moieties may also comprise immunodominant epitopes of vaccines including, for example, epitopes derived from diphtheria or tetanus toxin, influenza virus hemagglutinin, HBs antigen, hepatitis A or B virus, polio virus, rubella virus, measles virus, tuberculosis virus, and the like. Moreover, the haptenic moiety may comprise an alloantigen such as, for example, a fragment of a major histocompatibility antigen to which the host has been previously sensitized.

Conjugation of the therapeutic compound to the haptenic moiety results in the production of a "therapeutic compound-hapten conjugate". The therapeutic compound and the haptenic moiety may be covalently or non-covalently attached and may be joined directly through a chemical bond or through a bridge of not more than about 50 members in the chain, usually not more than about 20 members in the chain, where the members of the chain may be carbon, nitrogen, oxygen, sulfur, phosphorus, and the like. Thus, various techniques may be used to join the two members of the therapeutic compound-hapten conjugate, depending upon the nature of the members of the conjugate, the binding sites of the members of the conjugate, convenience, and the like. Functional groups that may be involved in the covalent conjugation of the members include esters, amides, ethers, phosphates, amino, hydroxy, thio, aldehyde, keto, and the like. The bridge may involve aliphatic, alicyclic, aromatic, or heterocyclic groups. The haptenic moiety may be "built into" the therapeutic compound during synthesis of that compound or may be conjugated to the therapeutic compound after that compound has been fully synthesized or

otherwise obtained. A substantial literature exists for combining organic groups to provide for stable conjugates.

In the case of therapeutic compounds which are peptides or proteins, the haptenic moiety may be conjugated to a reactive site on one or more of the amino acids which are present in the compound, either on a reactive side chain of an amino acid or at the C- or N-terminus of the peptide or protein. For example, amino acids such as lysine, arginine, glutamic acid, aspartic acid and others possess chemically reactive sites available for covalent conjugation to the haptenic moiety. Non-covalently linked conjugates may be prepared, for example, through biotin-avidin interactions, and the like. Conjugates involving only proteins or glycoproteins can be chimeric or fusion recombinant molecules resulting from expression of ligated open reading frames of natural sequences, synthetic sequences, or combinations thereof. The particular manner in which the haptenic moiety is joined to the therapeutic compound will not be critical to the invention so long as the haptenic moiety is available for binding to circulating antibodies in the vascular system.

In the embodiment of the invention which employs an α-Gal structure as the haptenic moiety, depending upon the nature of the chemistry, the α-galactosyl group may be conjugated to the therapeutic compound in a variety of ways. Various chemistries may be employed for joining the galactosyl group to a variety of functionalities. See, for example, Gobbo et al., *Int. J. Pept. Protein Res.* 40:54-61 (1992), Wood and Wetzel, *Bioconjug. Chem.* 3:391-396 (1992), Filira et al., *Int. J. Pept. Protein Res.* 36:86-96 (1990), Kazimierczuk et al., *Z. Naturforsch* 40:715-720 (1985), Rademann and Schmidt, *Carbohydr. Res.* 269:217-225 (1995) and Wong et al., *Glycoconj. J.* 10:227-234 (1993).

The number of therapeutic compounds conjugated to each haptenic moiety may vary. In some situations, it may be

desirable to have more than one therapeutic compound joined to each haptenic moiety to provide, for example, for a higher avidity between the conjugate and the target of interest. Generally, the number of therapeutic compounds conjugated to a haptenic moiety will be a function of the size and structure of the therapeutic compound and will usually be less than about 5, more usually less than about 3, frequently less than about 2 and most frequently 1. Moreover, in some instances, a higher ratio of haptenic moieties to therapeutic compounds may also be employed.

The subject Xenoject compounds can be used for the treatment of a wide variety of pathologies simply by varying the therapeutic compound employed. Thus, treatments may include such things as immunosuppression for organ transplantation, treatment of neoplasias such as carcinomas, leukemias, lymphomas, sarcomas, melanomas, and the like, hormonal therapy, treatment of bacterial or viral infection, etc.

The Xenoject compounds of the present invention will usually be administered to a mammalian subject as a bolus, but may be introduced slowly over time by infusion using metered flow, or the like. The Xenoject compounds will usually be administered in a physiologically acceptable medium, e.g. deionized water, phosphate buffered saline, saline, aqueous ethanol or other alcohol, plasma, proteinaceous solutions, mannitol, aqueous glucose, alcohol, vegetable oil, or the like. Other additives which may also find use include buffers, where the media are generally buffered at a pH in the range of about 5 to 10, where the buffer will generally range in concentration from about 50 to 250 mM salt, where the concentration of salt will generally range from about 5 to 500 mM, physiologically acceptable stabilizers, biocides, and the like, these additives being conventional and used in conventional amounts.

The Xenoject compounds described herein will for the most part be administered parenterally, such as intravascularly, intravenously, intraarterially, intramuscularly, subcutaneously, or the like, however, administration may also be orally, nasally, rectally, transdermally or inhalationally via an aerosol. Usually a single injection will be employed although more than one injection may be used, if desired. The Xenoject compounds may be administered by any convenient means, including syringe, trocar, catheter, or the like. The particular manner of administration will vary depending upon the amount to be administered, whether a single bolus or continuous administration, or the like. Often the administration will be intravascularly, where the site of introduction is not critical to this invention, preferably at a site where there is rapid blood flow so as to provide for systemic dissolution of the compound, e.g. intravenously, peripheral or central vein. The Xenoject compounds may also be administered locally so as to direct the compounds to a specific site.

Once administered to the mammal, the haptenic moiety of the therapeutic compound-hapten conjugate will be specifically recognized and bound by a circulating antibody present in the mammalian host. The circulating antibodies which serve to bind to and stabilize the Xenoject compound can be naturally occurring antibodies such as antibodies directed against, for example, blood group antigens, and the like, or anti-xenogenic antibodies. Antibodies that serve to bind to and stabilize the Xenoject compound may also be those induced in response to a prior presensitization of the mammalian host by, for example, a fragment of a major histocompatibility antigen, or in response to a prior vaccination of the mammalian host by, for example, diphtheria or tetanus antitoxin, influenza virus hemagglutinin, HBs antigen, hepatitis A or B virus, polio virus, rubella virus, measles virus, tuberculosis virus, etc. If desired, the host can be presensitized to the

particular hapten of interest prior to administration of the Xenoject compound.

The following examples are presented for illustrative purposes only and are not intended to be limiting of the invention described herein.

Experimental

Example 1

Improvement of Allotrap efficacy by prolongation of its in vivo half-life

Potent immunosuppressive peptides (amino acids 75-84) have been identified from the amino acid sequence of the human leukocyte antigen class I molecule. Clayberger and Krensky, *supra*. These peptides are known as Allotrap peptides and have been demonstrated to have immunosuppressive effects leading to the prolongation of organ allograft survival. Recent reports have demonstrated that there is a significant therapeutic advantage to effectively increasing the in vivo half life of Allotrap peptides by rendering those peptides resistant to hydrolysis by serum proteases. Gao et al., *supra*. This was accomplished by synthesizing the D-amino acid isomer of the natural peptide (D-Allotrap peptide).

Herein, we describe methods for effectively increasing the in vivo half-life of the D-Allotrap peptide through its incorporation into a Xenoject compound. The D-Allotrap peptide sequence was linked to the haptenic moiety Gal α1-3 Gal-NHS and tested in mice having naturally occurring circulating anti-α-Gal antibodies. The D-Allotrap/α-Gal Xenoject conjugates were demonstrated to bind to the circulating antibodies, survive for a significantly longer period in the circulation than do the unconjugated D-Allotrap peptides and have enhanced immunosuppressive effects as compared to the unconjugated D-Allotrap peptide.

Gal antigen, peptides and conjugates: The synthesis of the peptide-sugar conjugate first required the generation of an α-Gal disaccharide with a reactive group on the first carbon of the galactosyl ring. Briefly, this was accomplished by generating two bromine protected ring compounds (2,3,4, 6-tetra-O-benzyl-α-D-galactopyranosyl bromide and 4,6-O-benzylidene-1,2-O-isopylidene-α-D-galactopyranose). The two structures were joined in a sterically controlled synthesis to yield 2,4,6,2',3',4',6'-hepta-O-acetyl-3-O-α-D-galactopyranosyl-α-D-galactopyranosyl bromide. Treatment of the latter compound with a methanolic solution of sodium methoxide replaced the bromide with a thioglycoside of 3-thiopropionic acid. To complete the synthesis of a reagent permitting the incorporation of the α-Gal residue into a peptide, this structure was added to the secondary amine on the epsilon carbon of lysine (termed α-Gal-s-Lys where Lys is lysine). This was performed by first bubbling ammonium through buffer containing the alpha carbon protected amino acid (Sigma Chemical Co., St. Louis, Mo), the carbohydrate and carbonate, followed by the addition of a heterobifunctional linker N-hydroxy succinymidyl suberate.

The Allotrap D-B2702 amino acid sequence (Arg-Glu-Asn-Leu-Arg-Ile-Ala-Leu-Arg-Tyr) was then constructed with an automated peptide synthesizer (Synpep, Dublin, CA) such that the α-Gal-s-Lys was incorporated into the final peptide product. This resulted in a conjugate retaining its peptide immunosuppressive function that was capable of binding to anti-α-Gal antibodies.

α-galactosyltransferase knock-out mice: Mice were available that had an inactivated α-galactosyltransferase gene (αGalTKO mice: Thall et al., *supra*). The result was a mouse strain that did not express the terminal Gal α1-3 Gal sugar on its glycoproteins, and did develop a natural high-titer anti-α-Gal antibody response analogous to that seen in humans. The original B6D2 knock-out mice were back-crossed for six generations with C57BL/6 and DBA/2 mice in order to

establish the knockout gene on two syngeneic backgrounds. Eight week old male animals were used throughout the experiments.

Injection, sampling and cardiac transplantation: Allotrap peptides and Allotrap/α-Gal Xenoject conjugates were administered intravenously via the dorsal tail vein. Mice were anaesthetized with methoxyfluorane and blood was drawn via the retroorbital plexus every other day in order to follow serum levels of the products.

The survival of MHC mismatched heterotopic cardiac allograft transplants was monitored to assess the in vivo immunosuppressive effects of the free Allotrap peptide or of the Allotrap/α-Gal Xenoject conjugate. Briefly, hearts from adult male DBA/2 knock-out mice were transferred to the peritoneum of C57BL/6 αGalTKO mice according to the modified method of Ono and Lindsey, *supra*. Palpation of the transplanted hearts through the peritoneal wall permitted a direct evaluation of graft survival.

Peptide recovery from serum: Serum samples were taken as described above and assayed for the presence of D-Allotrap peptide by HPLC mass spectrophotometry. Previously, it had been shown that the D-Allotrap peptide resisted serum degradation much better than the natural L-amino acid variant. Gao et al., *supra*. Using the conditions worked out for that study, samples from αGalTKO mice were analyzed for the persistence of antibody bound D-Allotrap/α-Gal conjugate. Briefly, free peptides or conjugates were separated from the majority of globular proteins in the serum by heating to 55°C for 15 min in order to dissociate the immunoglobulin from the hapten. The sample was then passed over a C18 Sep-Pak (Millipore, Millford, MA) reverse-phase separation cartridge, and the peptides eluted with acetonitrile, 1% triethanolamine. The fraction purported to contain the peptide was then analyzed by HPLC and mass spectroscopy (Charles Evan's, Redwood City, CA).

Results and Discussion

Conjugation of the α-Gal residue to the D-Allotrap peptide was successful using the strategy described in materials and methods. Transplanted mice were treated for 10 days (1mg/Kg) with the unconjugated D-Allotrap peptide or with the D-Allotrap/α-Gal conjugate, and the survival of the transplanted hearts was compared to those from mice that received no treatment. As shown in Figure 1, the mice treated with unconjugated D-Allotrap peptide maintained their grafts for roughly 5 days longer (rejection on day 15) than did the untreated anneals. Strikingly, however, the mice treated with the D-Allotrap/α-Gal conjugate preserved their graft function for 25 days (rejection on day 35) beyond the untreated controls. This indicated that there was a significant advantage to conjugating the therapeutic peptide to a haptenic moiety.

Because the purported target for Allotrap peptide action may be an intracellular molecule (such as hsp 70; see Nossner et al., *supra*), it was unlikely that the enhanced activity of the D-Allotrap/α-Gal conjugate was due to redirection of the circulating antibodies to a specific target such that the immunoglobulins would then activate, complement and kill the cell. Instead, it was postulated that the therapeutic peptide was benefiting from a prolonged half-life in the serum when the haptenic moiety of the conjugate was bound to circulating serum immunoglobulins.

To determine if this was indeed the case, experiments were performed to examine the in vivo half-life of the conjugate as compared to that of the unconjugated peptide. A single large bolus (100 mg/Kg) of unconjugated D-Allotrap peptide or D-Allotrap/α-Gal conjugate was administered to αGalTKO mice (possessing circulating anti-α-Gal antibodies), or to normal antibody negative mice as a control. Blood was drawn from the animals, and selected samples were analyzed by HPLC and mass spectroscopy for the presence of the D-Allotrap peptide. The results are presented in Table I.

Table I

Recovery of D-Allotrap from Serum Following Intravenous Administration of the Free or α-Gal Conjugated Peptide to Antibody Positive or Negative Mice

Days	D-Allotrap αGalTKO	D-Allotrap Normal	D-Allotrap/α-Gal αGalTKO	D-Allotrap/α-Gal Normal
1	-	-	+	-
3	-	-	+	-
5	-	-	+	-
7	-	-	+	-
9	-	-	+	-
11	-	-	+	-
13	-	-	-	-
15	-	-	-	-

NB: (+) indicates that the D-Allotrap peptide was recovered from the serum sample, and (-) indicates that it was not.

As shown in Table I, unconjugated D-Allotrap peptide is not detectable even 24 hrs after administration to either antibody positive or negative mice. This corresponds to previous observations where the in vivo half-life of the Allotrap peptide was measured to be less than 60 min (Gao et al., *supra*). In contrast, however, the anti-α-Gal antibody positive mice injected with the D-Allotrap/α-Gal conjugate maintain the peptide in the circulation for approximately 11 days. No increased persistence of the conjugate in normal mice was observed. It is significant to note that if the samples were not heated to weaken the hapten immunoglobulin recognition, the peptide was not detectable. These results indicate that the peptide bound to the antibody copurified with the globular proteins. These results also indicate that the enhanced in vivo half life of the therapeutic compound (and, therefore, its prolonged therapeutic effect) was a result of binding of the peptide/hapten conjugate to circulating antibodies specifically directed against the hapten, thereby serving to stabilize the therapeutic compound in the vascular system.

All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method for prolonging the in vivo half-life of a therapeutic compound in a mammal, said method comprising:

conjugating said therapeutic compound to a haptenic moiety, thereby producing a therapeutic compound-hapten conjugate;

administering said therapeutic compound-hapten conjugate to said mammal, wherein the in vivo half-life of said therapeutic compound-hapten conjugate is prolonged as a result of binding to a circulating antibody present in said mammal.

2. The method according to Claim 1, wherein said therapeutic compound is selected from the group consisting of a peptide, a peptidomimetic, a protein, a nucleic acid, an organic molecule and a metallic molecule.

3. The method according to Claim 2, wherein said therapeutic compound is a peptide.

4. The method according to Claim 3, wherein said peptide is an Allotrap peptide.

5. The method according to Claim 4 wherein said Allotrap peptide is Arg-Glu-Asn-Leu-Arg-Ile-Ala-Leu-Arg-Tyr.

6. The method according to Claim 3, wherein said peptide is a cyclosporine.

7. The method according to Claim 1, wherein said therapeutic compound is interleukin-2.

8. The method according to Claim 1, wherein said therapeutic compound is a pro-drug that is hydrolyzed in vivo to produce a biologically active drug.

9. The method according to Claim 1, wherein said therapeutic compound is a pro-drug which is enzymatically cleaved in vivo to produce a biologically active drug.

10. The method according to Claim 9, wherein said pro-drug is pro-insulin.

11. The method according to Claim 1, wherein said haptenic moiety is a blood group antigen.

12. The method according to Claim 1, wherein said haptenic moiety is an immunodominant epitope of a vaccine.

13. The method according to Claim 12, wherein said haptenic moiety is an immunodominant epitope derived from a vaccine selected from the group consisting of diphtheria toxin, tetanus toxin, influenza virus hemagglutinin, hepatitis A virus, hepatitis B virus, polio virus, rubella virus and measles virus.

14. The method according to Claim 1, wherein said haptenic moiety is is an alloantigen to which said mammal has been presensitized.

15. The method according to Claim 14, wherein said alloantigen is a fragment of a major histocompatibility complex antigen.

16. The method according to Claim 1, wherein said haptenic moiety comprises galactose.

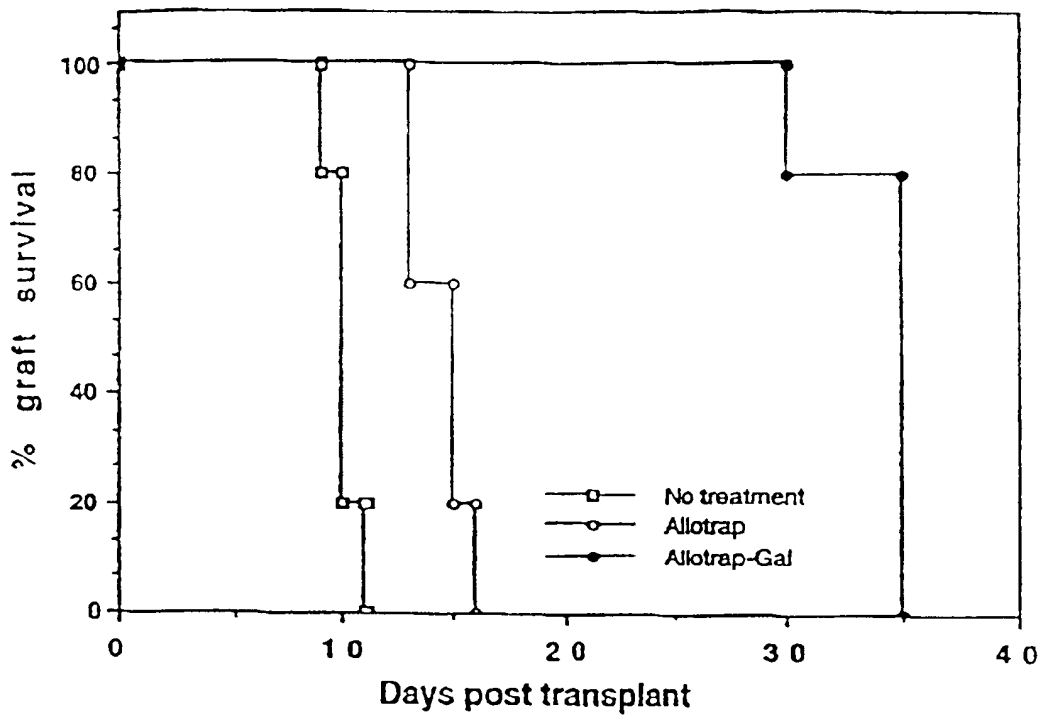
17. The method according to Claim 16, wherein said haptenic moiety is Gal α1-3 Gal.

18. The method according to Claim 1, wherein the haptenic moiety is a mimetic of the sugar moiety of a glycoprotein.

19. The method according to Claim 1, wherein said administering is orally, intravenously, subcutaneously or inhalationally.

20. A method for enhancing the therapeutic effect of a therapeutic compound in a mammal wherein said enhancing is the result of prolonging the in vivo half life of said therapeutic compound by the method of Claim 1.

FIGURE 1


% graft survival
100
80
60
40
20
0
0
10
20
30
40
Days post transplant
No treatment
Allotrap
Allotrap-Gal

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(81) Designated States: AU, CA, JP, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

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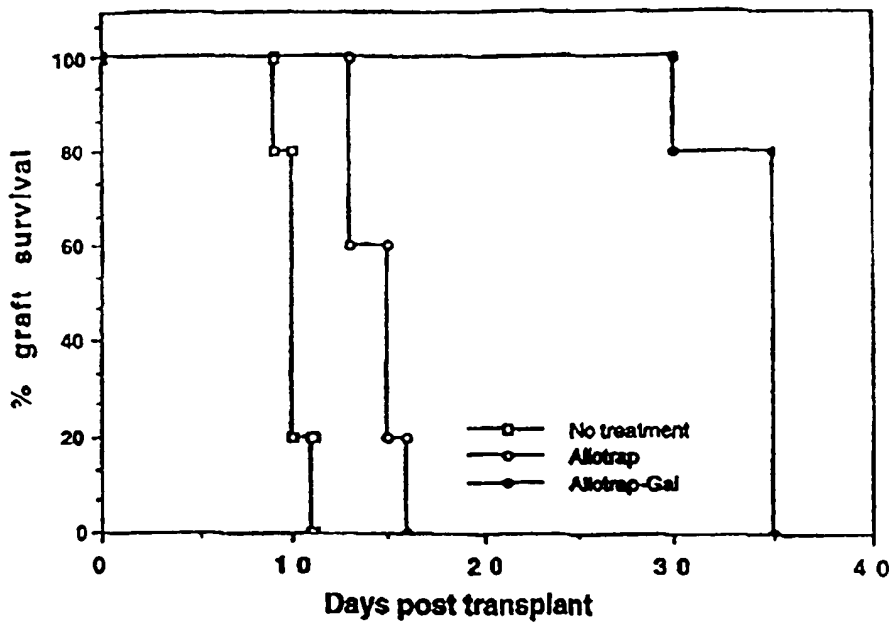

(57) Abstract

Novel methods for enhancing the effective in vivo half life of therapeutic compounds in mammals are provided. Specifically, therapeutic compounds are conjugated to a haptenic moiety. Upon administration to the mammalian subject, the therapeutic compound/hapten conjugates are bound by circulating antibodies directed specifically against the haptenic moiety of the conjugate, thereby resulting in a stabilization of the conjugate in the vascular system and an effective increase in the in vivo half life of the therapeutic compound.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/18475

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K47/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	LUSSOW A R ET AL: "REDIRECTING CIRCULATING ANTIBODIES VIA LIGAND-HAPTEN CONJUGATES ELIMINATES TARGET CELLS IN VIVO" JOURNAL OF IMMUNOTHERAPY: WITH EMPHASIS ON TUMOR IMMUNOLOGY, vol. 19, no. 4, July 1996, pages 257-265, XP002050531 cited in the application see page 264, paragraph 2 see page 263, column 2 see page 257, column 2 - page 258, column 1 --- -/--	1-3, 16-20

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
11 June 1998	08. OKT. 1998

Name and mailing address of the ISA	Authorized officer
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3

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/18475

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BUELOW R ET AL: "PROLONGATION OF SKIN ALLOGRAFT SURVIVAL IN MICE FOLLOWING ADMINISTRATION OF ALLOTRAP" TRANSPLANTATION, vol. 59, no. 4, 27 February 1995, pages 455-460, XP000652975 see abstract; figures 2-4 see page 458 ---	1-5
X	WO 96 35443 A (SANGSTAT MEDICAL CORP) 14 November 1996 see page 6, line 28 - page 7, line 2 see claim 1; table 1 ---	1-5, 16-20
X,P	WO 97 37690 A (SANGSTAT MEDICAL CORP) 16 October 1997 see page 8, line 32 - page 9, line 8; claims 1-7; examples 1-5 ---	1-3,14, 16-20
X,P	WO 97 24140 A (UNIV LELAND STANFORD JUNIOR) 10 July 1997 cited in the application see page 12 - page 13; claims 16,19,20,22 ---	1-5
X	WO 95 34321 A (UNIV LELAND STANFORD JUNIOR) 21 December 1995 see abstract see page 9, line 30 - page 10, line 5; claims 9-12,14 see page 6, line 15-30 ---	1-5
X	DE 42 38 416 A (MAX PLANCK GESELLSCHAFT) 19 May 1994 see claims 10-13; table 14 ---	1-5, 16-20
X	WO 93 17699 A (UNIV LELAND STANFORD JUNIOR) 16 September 1993 see page 15, line 15-20 see page 20, line 7-17 see page 41; claims 11,12,16; examples 14,15 -----	1-5

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INTERNATIONAL SEARCH REPORT

In ational application No
PCT/US 97/ 18475

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.
because they relate to subject matter not required to be searched by this Authority, namely
See FURTHER INFORMATION SHEET in annex

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1° Subj.: 3-5, part of 1,2,14,16-20
2° Subj.: 6, part of 1,2,14,16-20
3° Subj.: 7, part of 1,2,14,16-20
4° Subj.: 8,9,10, part of 1,2,14,16-20
5° Subj.: 11, part of 1,2,14,16-20
6° Subj.: 12,13, part of 1,2,14,16-20
7° Subj.: 15, part of 1,2,14,16-20

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

3-5, part of claims 1,2,14,16-20

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest

[ ] No protest accompanied the payment of additional search fees

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Although claims 1-20 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Further defects under Article 17(2)(a):

Claims Nos.: 1, 2

In view of the large number of compounds, which are defined by the general definition in the indipendent claims, the search had to be restricted for economic reasons. The search was limited to the compounds for which pharmacological data was given and/or the compounds mentioned in the claims, and to the general idea underlying the application. (See Guidelines, Chapter III, paragraph 2.3).

INTERNATIONAL SEARCH REPORT

formation on patent family members

International Application No
PC., US 97/18475

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